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Award Number: DAMD17-01-1-0410

TITLE: Molecular Characteristics of Multicorn, a New Large Proteolytic Assembly and Potential Anti-Cancer Drug Target, in Human Breast Cancer Cells

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REPORT DATE: May 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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20021101 068

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2002	Annual (1 May 01 - 30 Apr 02)

4. TITLE AND SUBTITLE
Molecular Characteristics of Multicorn, a New Large Proteolytic Assembly and Potential Anti-Cancer Drug Target, in Human Breast Cancer Cells

5. FUNDING NUMBERS
DAMD17-01-1-0410

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Proper regulation of cell division and differentiation are major factors preventing neoplastic growth, and proteolysis is one of controlling mechanism of these processes. We have discovered a new giant proteolytic complex ubiquitous among Eukaryotes. The enzyme named multicorn apparently takes part in cell cycle regulation and is involved in partial overcoming the physiological effects of inhibitors targeting a well-known drug target, proteasome . The multicorn may constitute an attractive target for anti-cancer drugs, a marker for neoplastic transformation and may influence success of drugs targeting proteasome. The studies conducted lead toward understanding biological role of this enzyme. Our findings up to date: (i) the multicorn, which was first found in the cytosol, is present in the nucleus of mitotic cells. Importantly, it is detectable only in nucleus of the control MCF-10A breast cells, and not in the MCF-7 cancerous cells. (ii) The human multicorn is built from a single subunit. The oligomerization and activity of multicorn apparently is regulated by phosphorylation of this subunit in several distinct sites. (iii) The pattern of phosphorylation is specific for the particular cellular fraction, and differs between nonsynchronous, mitotic and overconfluent cells, and between control and cancerous cells.

14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)
controlled proteolysis, cell cycle regulation, cell proliferation, large enzymatic assemblies, protein biochemistry

15. NUMBER OF PAGES
15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Proper regulation of cell division and cell differentiation are the major factors preventing the neoplastic growth. Proteolysis is one of the widely accepted controlling mechanisms of these processes. Transcription factors and cell cycle regulatory proteins have to be activated or removed in a precise and timely fashion during the life of the cell. To a very large extent controlled proteolysis in cytosol and nucleus is executed by the giant multifunctional enzyme named the proteasome. The proteasome is an acknowledged anti-cancer drug target and one of specific inhibitors of the proteasome recently successfully finished phase II clinical trials against multiple cancers, including breast cancer. We have discovered a new giant proteolytic complex distinct from the proteasome and ubiquitous among Eukaryotes. The enzyme named multicorn apparently takes part in the cell cycle regulation and is involved in partial overcoming the physiological effects of proteasome inhibitors. Up to date, we found significant differences in the activity, amount, oligomerization status, posttranslational modifications and subcellular localization of the multicorn in human breast cancer MCF-7 cells, as compared with non-cancerous MCF-10A cells. We monitored the activity, composition and distribution of the multicorn on different stages of the cell cycle in the two cell lines. We discovered that, similarly to the proteasome, the multicorn can be present in the nucleus and can be associated with the subcellular membrane, most probably on the outside of the endoplasmic reticulum. Significantly, the nuclear localization is especially apparent in mitotic cells and is clearly connected with the phosphorylation of the multicorn subunit. Since mitosis is the period of greatest vulnerability of cells to anti-cancer drugs, the multicorn can be considered an attractive potential drug target alone or in combination with the proteasome. Our findings of sharp differences in the multicorn physiology between the cancerous and non-cancerous cell lines confirm that the multicorn can be also a useful marker of neoplastic transformation. We will continue our studies on the role of multicorn in cell cycle regulation and on mechanisms underlying the regulation of the multicorn activity.

BODY

Up to date, the research accomplishments associated with the objectives and tasks outlined in the approved Statement of Work are as follows:

Objective 1. Cloning and expressing the gene of human multicorn monomer.

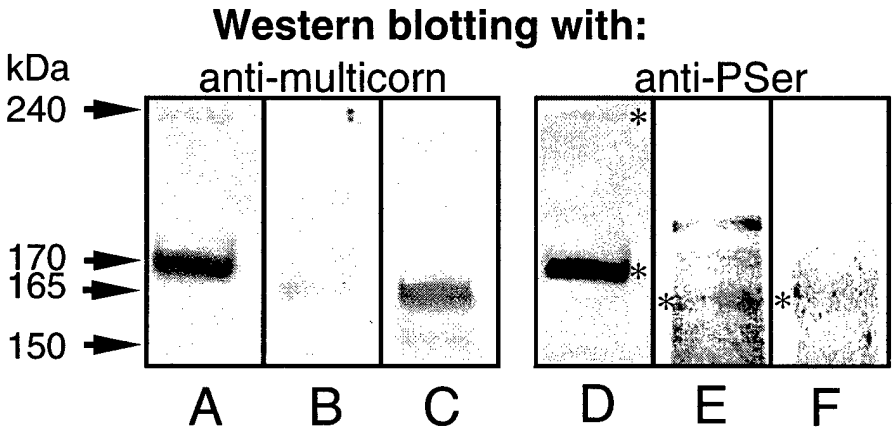
***Task 1**: months 1 - 12; molecular basis of different physical and chemical properties of the two multicorn subunits will be studied using a combination of peptide mapping, sequencing and mass spectroscopy.*

The task is near completion. We established that the human multicorn is built from a single subunit, similarly to the previously described by our group multicorn from fission yeast (*Schizosaccharomyces pombe*). The single subunit has apparent molecular mass 150 kDa. The 150 kDa subunit can be phosphorylated on several distinct serine residues to render polypeptides of electrophoretic mobility 165 kDa, 170 kDa and 240 kDa.

In our preliminary data we described two multicorn subunits: of apparent molecular mass of 150 kDa and 170 kDa. We found that the two subunits built a large oligomer, named "large form", of apparent molecular mass about 4,000 kDa, as approximated by gel filtration chromatography.

Up to date, we confirmed the presence of the 150 kDa and 170 kDa (sometimes resolvable to doublet) in the large form of the multicorn. We established that both subunits react with polyclonal antibodies raised against the 150 kDa subunit of the fission yeast multicorn when tested by Western blotting (Fig. 1a). Additionally, we found new bands reacting with anti-multicorn antibodies on Western blots: 165 kDa and 240 kDa (Fig. 1). The 165 kDa, 170 kDa and 240 kDa bands reacted with monoclonal anti – phosphoserine antibodies (Fig. 1d, e,). Analysis of the Western blots probed with four different anti-phosphoserine antibodies (Calbiochem) recognizing phosphoSer in specific environment of amino acid residues indicated that the 150 kDa polypeptide is most likely phosphorylated on distinct serines to produce the three modified forms. Antibodies against anti-phosphotyrosine and anti-phosphothreonine failed to recognize the multicorn subunits. Currently, we are in the process of purification of the multicorn subunits to determine the sites of phosphorylation and the extent of the modification with mass spectroscopy. The pattern of phosphorylation is far from a simple "one modification site" model. This makes the multicorn control even more exciting, taking into account the regular, cell cycle - dependent pattern of changes in subcellular localization of differently modified multicorns we described below.

Fig. 1 Multicorn subunits of different electropho-retic mobility are pho-sphorylated in distinct manner. Showed are Western blottings of diffe-rent subcellular fractions of MCF-7 or MCF-10A cells probed with specific anti-multicorn antibodies (A-C) or antibodies against phos-phoserine (D-F). A and D-cytosol, MCF-10A; B and E -membrane fraction, MCF-7; C and F - membrane fraction, overconfluent MCF-7; Stars designate the multi-corn bands in blots probed with anti-PSer antibodies.



Task 2: months 1 - 6; cloning the gene, or genes, encoding the human multicorn monomers using HeLa cDNA library and PCR technology. It will be determined if the two types of subunits are encoded by the same gene.

We established that the 150 kDa and 170 kDa subunits have to be encoded by the same gene, since the 170 kDa polypeptide is a posttranslationally modified form of the 150 kDa protein (see task 1).

The cloning of the gene encoding the multicorn subunit is in progress and is expected to be finished within 6 months. The delay in realization of this task was caused by a temporary shift of

focus from Objective 1, Task 2, to Objective 3, Task 1. The temporary shift was prompted by new results obtained with *S. pombe* multicorn and during the work on Objective 2 of this study (Osmulski and Gaczynska, 1998). First, we analyzed in – depth the fission yeast multicorn knockout mutant. The mutant was viable, however with a strong phenotype which correlates with cell cycle dependent changes in the multicorn properties. Our most striking finding was the acute defect in sister chromatid separation in anaphase. Instead of typical for anaphase B clearly separated daughter nuclei on the two ends of a mitotic cell, we often observed long "threads" stained with DAPI in the phase immediately proceeding G1/S. This observation confirmed the postulated role of the eukaryotic multicorn in cell cycle progression (Osmulski and Gaczynska, *in preparation*). Second, we found that in fission yeast cell cycle dependent changes in total amount of multicorn, its activity, aggregation state, level of phosphorylation, and cellular localization. Third, when working on the Objective 2 of this study, we found that the multicorn is also present in the nucleus in a small portion of the non-synchronous cells. These important findings encouraged us to probe the cell – cycle dependent changes in the multicorn localization, activity and modifications. Additional justification for the temporary shift between Objectives is that the results obtained during our cell cycle – related studies (see Objective 3) are pivotal for the efficient purification and characterization of the distinct multicorn forms in Objective 2.

Task 3: *months 6 - 18; expressing the gene(s) in Schizosaccharomyces pombe or mammalian expression system. The goal of this task is to obtain active recombinant multicorn molecules characterized by controlled content of the specific monomers.*

The work on this task will begin as soon as the cloned gene encoding the multicorn subunit will be available. The cause for the temporary delay in realization of this task is justified above (see Objective 1, Task 2).

Objective 2. Studying the mechanism controlling the multicorn activity through its oligomerization and phosphorylation.

Task 1: *months 6 - 24; The multicorn complexes of different supramolecular organization and posttranslational modifications will be separated and purified. The qualitative and quantitative parameters of proteolysis catalyzed by these distinct complexes will be determined using selected protein substrates and model fluorogenic peptide substrates.*

A significant part of this task has been already completed. We found that the large form of the multicorn (about 4,000 kDa) is built mostly from the 170 kDa and small amounts of the 165 kDa and 240 kDa phosphorylated forms of the 150 kDa multicorn subunit. We found that the small form of the multicorn (900 kDa) is assembled mostly from the phosphorylated 240 kDa and non-phosphorylated 150 kDa polypeptides, and trace amounts of the phosphorylated 170 kDa protein. We separated the large and small forms of the multicorn, and determined their specific activities with a model fluorogenic peptide substrate.

On the basis of our experience accumulated during research devoted to the multicorn from fission yeast, we expected to find both the large (about 4,000 kDa) and the small (900 kDa)

forms of the proteolytic complex in MCF-7 and MCF-10A cells. Indeed, we confirmed the presence of both the oligomeric forms in the cytosol of both types of cells. We purified the two forms by a set of differential centrifugations combined with gel filtration and anion exchange chromatography (Gaczynska et al., 1993; Glas et al, 1998). Specifically, we enriched the cytosolic fractions of the cells with the large protein assemblies using 5 – hour ultracentrifugation at 100,000xg (at 4°C). The resulting protein pellet was resolubilized and subjected to gel filtration chromatography on a Superose 6 column (Pharmacia), especially designed for the separation of the large biomacromolecules with high resolution (Osmulski and Gaczynska, 1998). The applied procedure produced fractions containing about 80% pure large form (eluted at 6.5 – 7.5 ml), and fractions containing about 20% of the small form (eluted at 9.5 – 11 ml, Fig. 2). Further purification of the multicorn was carried out by anion exchange chromatography on the HQ/M column (Applied Biosystems). Identity of the isolated oligomeric forms was confirmed on Western blots probed with the specific polyclonal antibodies raised against the fission yeast multicorn.

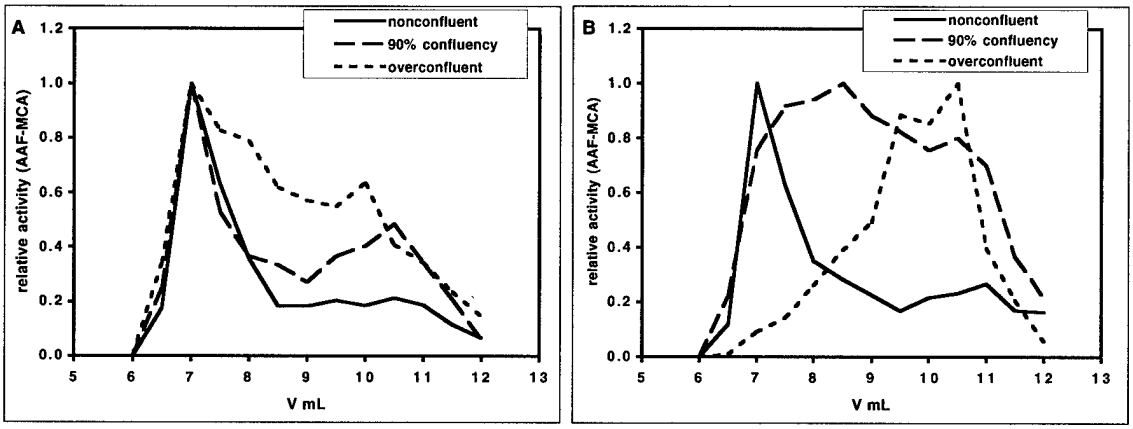


Fig. 2. Gel filtration profiles of peptidase activity measured with AAF-MCA in 5hr pellet fractions of (A) MCF-7 and (B) MCF-10A. The cells were harvested as non-confluent, subconfluent (90%) or overconfluent. The activity profiles show presence of two major forms of the multicorn: the large form is eluted at 6.5-7.5 mL and small form at 9.5-11 mL. The increase of cell culture density shifted the equilibrium between forms from the dominance of the large form to the dominance of the small form in MCF-10A cell. MCF-7 cells show a very limited shift under similar conditions. The activities are standarized for each profile against the highest value of activity in the profile.

We found that the two oligomeric forms in the cytosols of both MCF-7 and MCF-10A cells clearly differ in the phosphorylation pattern of their subunits. Namely, the large form of the multicorn contained mostly the 170 kDa subunit and small amount of the 165 kDa and 240 kDa phosphorylated forms of the 150 kDa subunit. In the small form we found mostly the phosphorylated 240 kDa and non-phosphorylated 150 kDa polypeptides, and only trace amounts of the phosphorylated 170 kDa protein.

In the non-confluent and nonsynchronous MCF cells most of the multicorn - associated proteolytic activity is linked with the large oligomeric form. As we found, the specific activity of the large form in the cytosol of control MCF-10A cells is about 6 – fold higher than in the cytosol of cancerous MCF-7 cells (Fig. 3).

Moreover, the multicorn large forms isolated from the two cell lines differed about two - fold in their content of the 170 kDa phosphorylated subunit (Fig. 3). We suspect that the 170 kDa subunit is responsible for most of the activity detected with the model peptide substrate, AlaAlaPhe-methylcoumarin (AAF-MCA). Still, the two - fold difference in the protein content could hardly account for the six – fold difference in the multicorn activity. There are several probable explanations for this finding. It is possible that the 170 kDa subunit plays slightly different roles in the large forms resulting in their distinct specific activities. The large forms, although eluted at the same volume, are likely organized differently in the two cell lines. The reason for the detection of very little of the multicorn activity in the MCF-7 cells may lay in its lower stability. Obviously, some other factors, perhaps the content of other, differently phosphorylated subunits, may also modulate the specific activity of the large form. Therefore, it is imperative to broaden the scope of these studies and carefully determine specific activities of the large forms isolated from both cell lines and composed from distinct amounts of 170 kDa and 165 kDa subunits. We already established conditions, which allow us to obtain analytical quantities of the differently modified complexes (see Objective 3). We determined that the ratio of large and small forms of the multicorn dramatically differed in the cytosols of nonconfluent and overconfluent MCF-10A cells. The large form was predominant in nonconfluent cells, whereas the small form was the only detectable active form of the multicorn in the cytosol of overconfluent (G0) cells (Fig. 2). The correlation between the cell density and the multicorn oligomerization status was almost completely absent in cancerous MCF-7 cells. Here, the large cytosolic form was always predominant with only a small decline in the overconfluent cell culture (Fig. 2)

A complete characterization of the mechanisms governing dependence of specific activity upon the subunit composition and modifications of the multicorn oligomers in both the cell lines is pending. Since we already found the conditions for obtaining the large amounts of the small form by simply overgrowing the MCF-10A cells (Fig. 2) the supply of this form should not hinder our experiments.

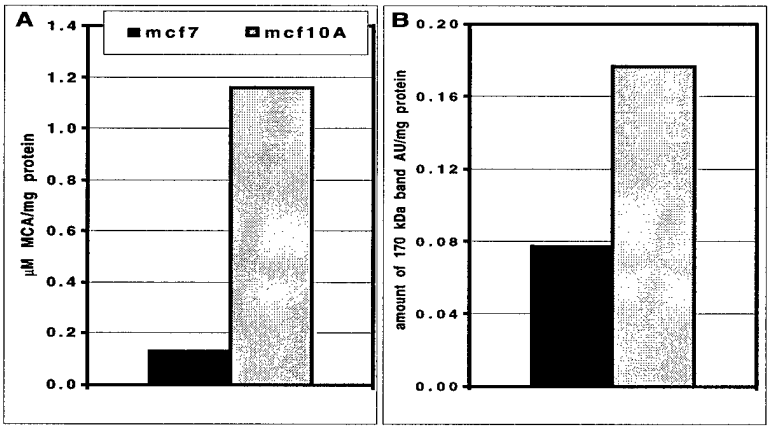


Fig. 3. Specific peptidase activity of multicorn is six fold higher in cytosol of MCF-10A cells than in MCF-7 cells (A). This observation is in part explained by the fact that amount of the 170 kDa subunit/mg protein in cytosol is more than two fold higher in MCF-10A than MCF-7 cells (B).

Objective 3. Molecular characterization of the multicorn at different stages of the cell cycle. *Armed with our experience with the fission yeast multicorn, we will test how the activity, oligomerization status and posttranslational modifications of the multicorn change during cell cycle progression in human breast cancer MCF-7 cells and non-cancerous breast cells MCF-10A.*

***Task 1**: months 12 - 18; we will perform flow cytometric analysis of nonsynchronous MCF-7 and MCF-10A cells stained with anti-multicorn antibodies and with propidium iodide (DNA).*

The work on this task is has just started, according to the schedule.

***Task 2**: months 18 - 36; we will analyze the expression and biochemical properties of the multicorn in synchronized MCF-7 and MCF-10A cells.*

A part of the task has been already completed and yielded very important results. As explained in the progress reports for Objective 1, we decided to temporary shift our focus toward characterization of the biochemical properties of the multicorn in cell-cycle synchronized cells. We discovered that the subcellular localization of the multicorn depends on the cell cycle stage: the multicorn was detectable in the nucleus only during mitosis, and only in the control MCF-10A cells. We found that the pattern of differently phosphorylated subunits is specific for the particular cellular fraction, and differs between nonsynchronous, mitotic and overconfluent (G0) cells. These findings, especially the nuclear localization of the protease in the control but not cancerous cells, may hold the key to revealing the potential biological role of the multicorn in breast cancer.

We analyzed the multicorn subunit composition in nonsynchronous, nonconfluent cells, overconfluent cells and cells arrested in metaphase by treatment with 15 micrograms /ml of nocodazole for 15 hours (Wang et al., 1997). The cells were fractionated into cytosolic, nuclear and membrane fractions by a following procedure. The cells suspended in RSB buffer (10 mM Hepes pH 6.2, 10 mM NaCl, 1.5 mM $MgCl_2$) were broken by 80 strokes of a Dounce homogenizer. The pellet obtained after centrifugation of the lysate (3 min, 2,500xg, with additional washing of the pellet) was resolubilized in RIPA buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, 0.5% IgepalCA-630) and constituted the nuclear fraction. The supernatant was subjected to 1.5 – hour ultracentrifugation at 100,000xg. The resulting supernatant after the ultracentrifugation was saved as the cytosolic fraction. The remaining pellet after the resolubilization in RIPA buffer yielded the membrane fraction. All procedures were carried out at 4°C, and all buffers were supplemented with a protease inhibitors and phosphatase inhibitors cocktail (PMSF, aprotinin, leupeptin, pepstatin, NaF, $NaVO_4$). The fractionation of the cells executed without the inhibitors led to the formation of additional bands of lower molecular weight, especially 150 kDa detectable with SDS-PAGE. In general, they still followed the described polypeptide pattern, with some proteolytic degradation apparent on Western blots. Interestingly, the pattern of subunits in MCF-7 cells was much more sensitive to the lack of the inhibitors than in MCF-10A cells suggesting possibly higher dynamics and faster turnover of the multicorn in the cancerous cells.

The details of the subunit patterns observed under the different culture conditions are summarized in Tables 1 and 2 and are shown in Fig. 4. The major findings are summarized below.

(a) We confirmed the presence of the 150, 165, 170 kDa (sometimes resolvable to doublet), and 240 kDa polypeptide bands on Western blots probed with polyclonal anti-multicorn antibodies;

(b) the polypeptides of apparent molecular masses 165, 170, and 240 kDa were found to be anti phosphoSer responsive;
(e) the observed pattern and content of subunits differed between MCF-7 and MCF-10A cell lines;
(d) the multicorn was detected not only in cytosol but also for the first time in the nuclear and membrane fractions;
(e) the pattern of major multicorn polypeptides was specific for the particular cellular fraction in the non-synchronized cells;
(f) the subcellular localization of the multicorn in the MCF-7 and MCF-10A cells was distinct;
(g) localization of the multicorn depended on a cell cycle stage: subcellular fractionation of the nocodazole synchronized culture revealed that multicorn was detectable in the nucleus only during mitosis.

On the basis of the collected data we may draw the following conclusions:
- only mitotic MCF-10A cells show the 170kDa band in nucleus;
- besides nucleus, the 170kDa band is commonly present in MCF-10A cells;
- the 240 kDa band is always cytosolic in both cell lines;
- the 165 kDa band can be membrane or cytosolic in both cell lines;
- 165 kDa band is commonly present in MCF-7 cells instead of 170 kDa band;
- MCF-7 cells show the presence of 170 kDa band only is cytosol of the overconfluent culture;
- no multicorn was detected in nucleus of MCF-7 cells;
- 150 kDa band (nonphosphorylated) is found only in membranes and cytosol of overconfluent MCF-7 cells;
- 170 kDa band is always very strong in cytosol of MCF-10A.

Table 1 The pattern of multicorn subunits differs between subcellular compartments, between cells in distinct physiological state and between the cancerous and control.

Culture conditions	Cellular fraction	MCF-7 (kDa)	MCF-10A (kDa)
Not synchronized	Cytosolic	240, 165	170, 165
	Membrane	165	170 closely spaced doublet
	Nuclear	none	none or traces of 170 kDa band
overconfluent	Cytosolic	240, 170, 150	240, 170
	Membrane	165, 150	170, 165
	Nuclear	None	None
mitotic	Cytosolic	240, 165	240, 170
	Membrane	165	170 closely spaced doublet
	Nuclear	none	170

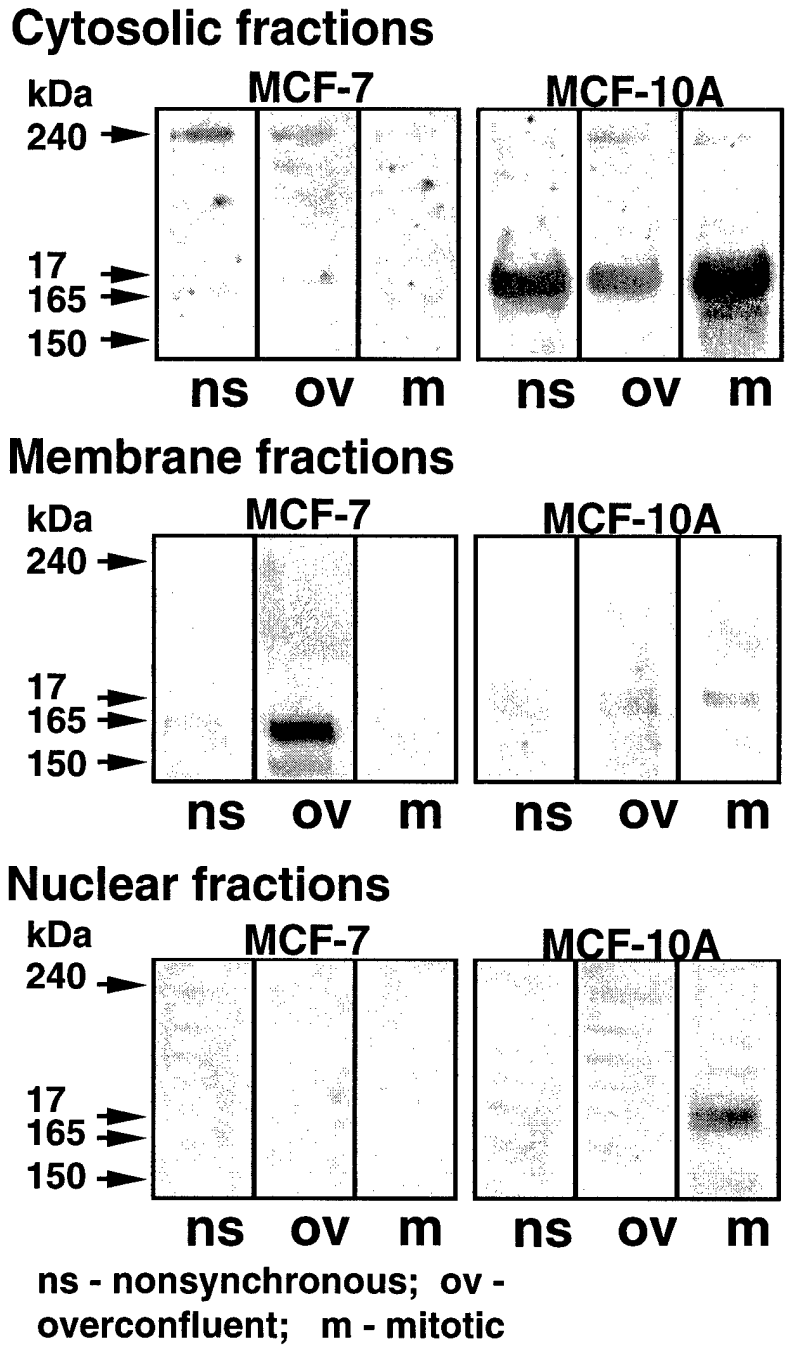


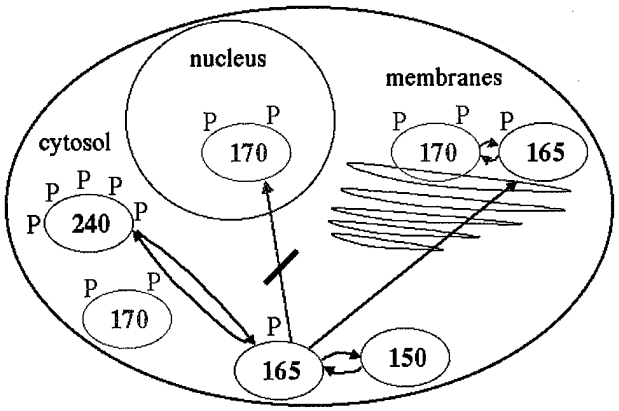
Fig. 4 The pattern of multicorn subunits differs between subcellular compartments, between cells in distinct physiological state and between the cancerous and control cells (see Tables 1, 2 . and text for details). Cytosolic, membrane and nuclear fractions were subjected to SDS-PAGE (6% acrylamide) and Western blotting, and the blots were probed with specific anti-multicorn antibodies.

Table 2 The major differences between localization of multicorn in control and cancerous breast cells.

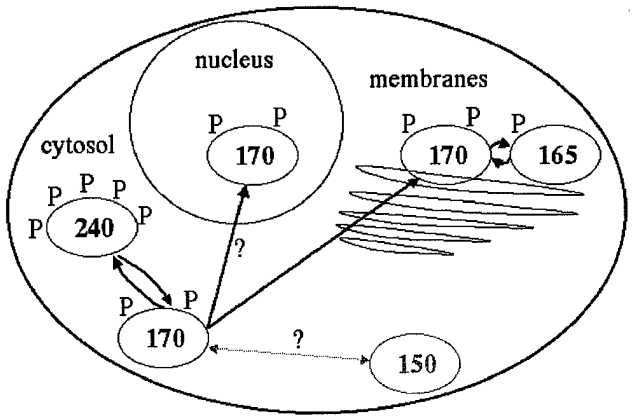
THE MAJOR DIFFERENCES	MCF-7	MCF-10A
multicorn present in nucleus during mitosis	-	+
strong 170 kDa band cytosol	-	+
strong 165 kDa band in overconfluent membranes	+	-
overall band pattern similar in non-synchronous and mitotic cells	+	-

On the basis of the data we propose the following hypothetical model of localization and modifications of the multicorn in MCF-7 and MCF-10A cells.

MCF7



MCF10A



In the case of the MCF-10A cells the most of the multicorn resides in the cytosol as the large form built from 170 kDa phosphorylated polypeptide with a presumably over phosphorylated minor component of 240 kDa. A small portion of the multicorn is also bound to ER membranes mostly as 170 kDa doublet and 165 kDa phosphopolypeptide. The biochemical basis for the formation of the 170 kDa doublet remains at this moment unknown. The membrane-localized multicorn probably plays a distinct role from the cytosol-confined protease since it is specifically localized to this compartment in the overconfluent cells .

At a certain point before metaphase the multicorn enters the nucleus and remains in it for the most of this stage. The total amount of the multicorn increases during mitosis followed by the increase of its phosphorylation. Upon the completion of the mitosis the multicorn is probably destroyed by unknown pathway with the possible signaling through overphosphorylation.

The total amount of the detectable multicorn is much lower in cytosol of MCF-7 cells. The 240 kDa band constitutes the major cytosolic polypeptide building the large form. It is possible that the 165 kDa band also participates in the process of formation of the large form. The multicorn has never been detected in nucleus in these cells. It is unclear if the protease is very short lived in MCF-7 cells, there is a block in its transport, (again, most likely via phosphorylation) or there is a problem with the multicorn assembling in the nucleus. Interestingly, the 165 kDa band is the most prominent polypeptide in the membrane fraction creating a huge deposit of membrane bound multicorn during G0 phase. The almost complete lack of the 170 kDa subunit suggests that only in that form the polypeptide can be transported to the nucleus. The 150 kDa band representing the non-phosphorylated subunit is detectable only in MCF-7 cells what indicates either overproduction of the subunit or in more general terms its deficient turn-over.

Apparently, the process of formation of the large form, the multicorn transport and its fate depends on the level of its phosphorylation.

Despite challenges arising in attempts to explain the biochemical basis of these complicated observations they confirm the previous suggestions that the regulation of multicorn activity could be an important part of mechanism of the cell division.

KEY RESEARCH ACCOMPLISHMENTS

- We established that the human multicorn is built from a single subunit of apparent molecular mass 150 kDa. We found that the 150 kDa subunit can be phosphorylated on serine residues in several distinct sites to render polypeptides of electrophoretic mobility 165 kDa, 170 kDa and 240 kDa.

- We discovered that the multicorn, which was first found and described in the cytosol, is present in the nucleus of mitotic cells. Importantly, the multicorn is detectable only in nucleus of the control MCF-10A cells, and not in the MCF-7 cancerous cells.

- We established that the pattern of differently phosphorylated subunits is specific for the particular cellular fraction, and differs between nonsynchronous, mitotic and overconfluent (G0) cells. The pattern in specific subcellular compartments differs between control and cancerous cells.

- We isolated the large and small oligomeric forms of the multicorn and determined their subunit composition in respect to differently phosphorylated polypeptides.

- We found that the large oligomeric form of the multicorn isolated from MCF-10A control cells exhibits several - fold higher specific activity toward a model peptide substrate than the large form isolated from breast cancer cells MCF-7. This difference is accompanied by a distinct pattern of subunit phosphorylation.

- We found that the ratio of large and small forms of the multicorn dramatically differ in cytosols of nonconfluent and overconfluent control MCF-10A cells. The large form was predominant in nonconfluent cells, whereas the small form was the only detectable active form of the multicorn in the cytosol of overconfluent (G0) MCF-10A cells. To the contrary, in cancerous MCF-7 cells the large cytosolic form was always predominant with only a small decline in overconfluent cell culture.

REPORTABLE OUTCOME

1. The research conducted on the project will be a topic of the poster on 12th Annual Symposium on Cancer Research in San Antonio (July 12th, 2002). The abstract for the poster: **"Cancer, proteases and proteolytic instability."** by Pawel A. Osmulski, Xianzhi Jang, Bingnan Gu and Maria E Gaczynska, have been submitted and is attached in APPENDICES.

2. Two research assistants, Cynthia Galindo, B.S. and Edward Trevino. B.S., both Hispanics, were/are trained in biochemical and cell biology techniques as a part of conducted research. Mr. Edward Trevino is going to continue his education as a medical graduate student.

3. Graduate student in Molecular Medicine, Bingnan Gu, was trained in a variety of biochemical techniques when rotating in PI's laboratory. His work on the multicorn constituted a part of the abstract submitted to the 12th Annual Symposium in Cancer Research in San Antonio (attached).

CONCLUSIONS

Our results show that breast cancer MCF-7 cells posses a distinct regulation of proteolysis executed by the multicorn when compared with non-cancerous MCF-10A cells. The apparent lack of the multicorn in the nucleus of MCF-7 cells may constitute an important link between the overall efficiency of cell division and nuclear proteolysis. The cellular distribution of the multicorn, similarly to the proteasome, is not limited to cytosol, however the most of the both proteases resides in this compartment. Regulation of the assembly of the large and small forms of multicorn is accomplished through a complex phosphorylation pattern of their subunits. Moreover, it seems that the phosphorylation also controls subcellular localization of the multicorn and ultimately its fate.

On the basis of our data we may envision that multicorn constitutes an important player in cellular protein turnover probably also involved in regulation of cell cycle. Its distinct properties in the control and cancerous cells suggest that the multicorn may represent an attractive drug target and a marker of physiological state of the cells.

Future studies should indicate whether the multicorn participate directly in cell cycle regulation. The results of studies on the fission yeast homologue of the protease strongly support that possibility.

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Wang, S.-C., Lin, S.-H., Su, L.-K., Hung, M.-C. (1997) Changes in BRCA2 expression during progression of the cell cycle. Bioch. Bioph. Res. Comm. 234, 247-251.

APPENDICES

1. Abstract submitted for the 12th Annual Symposium in Cancer Research in San Antonio (July 12, 2002, San Antonio, TX)

Cancer, proteases and proteolytic instability. *Pawel A. Osmulski, Xianzhi Jang, Bingnan Gu, Maria E Gaczynska. Department of Molecular Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX 78245.

Protein degradation, in concert with protein synthesis, governs the proper execution of metabolic processes in the cell. The large, intracellular proteases like anti-cancer drug target proteasome, a novel protease multicorn, tripeptidyl peptidase II (TPPII) or leucine aminopeptidase (LAP) play a key role among all proteolytic enzymes due to their diverse functions. The proteasome actions are essential for cell cycle regulation, turnover of transcription factors and antigen processing. Inhibition of proteasome leads to cell death and is utilized to kill tumor cells. However, there are strong indications that the other proteases may also constitute valuable anti-cancer drug targets. The postulated duties of multicorn include degradation of cell cycle related factors and, together with TPPII and LAP, further processing of antigenic peptides produced by proteasome. The collaboration of proteasome and other large proteases suggests the existence of a net of functional relationships between the executors of controlled proteolysis. We demonstrate here that proteolytic instability, which manifests in changing the equilibrium between the activities of large cytosolic proteases is one of the signs of neoplastic transformation in human breast cancer MCF7 cells, as compared with non-cancerous MCF10A cells. In the functional proteomics fashion we found prominent changes in activities of the enzymes, in their subunit composition and subcellular distribution. Specifically, we found that there is markedly less proteasomes in nuclei of the cancerous than control cells, and nuclei of cancer and control cells have dramatically different pattern of subunits of a natural proteasome activator. Since proteasome, multicorn, TPPII and LAP all take part in antigen processing, the changes may impair removal of transformed cells by the organism. Probing the role of large proteases in maintaining the proper advance of cell cycle we discovered that the predominantly cytosolic multicorn can be found in the nucleus. Interestingly, both nuclei and cytosol of cancer cells contain mostly overphosphorylated, unstable forms of the protease. We postulate that the nuclear localization of multicorn holds the key for dissecting its role in cell cycle progression.